



Sequence Listing

<110> Se-Chang KWON
Sung-Youb JUNG
Sung-Min BAE
Gwan-Sun LEE

<120> Modified human granulocyte-colony stimulating factor and process for producing same

<130> PCA00729/HMY

<140> US/10/031,123

<141> 2002-01-09

<160> 79

<170> KOPATIN 1.0

<210> 1

<211> 522

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(522)

<400> 1

aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1

5

10

15

tgc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20

25

30

gag aag ctg tgt gcc acc tac aag ctg tgc cac ccc gag gag ctg gtg 144
 Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
 35 40 45

ctg ctc gga cac tct ctg ggc atc ccc tgg gct ccc ctg agc tcc tgc 192
 Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
 50 55 60

ccc agc cag gcc ctg cag ctg gca ggc tgc ttg agc caa ctc cat agc 240
 Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
 65 70 75 80

ggc ctt ttc ctc tac cag ggg ctc ctg cag gcc ctg gaa ggg ata tcc 288
 Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
 85 90 95

ccc gag ttg ggt ccc acc ttg gac aca ctg cag ctg gac gtc gcc gac 336
 Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
 100 105 110

ttt gcc acc acc atc tgg cag cag atg gaa gaa ctg gga atg gcc cct 384
 Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
 115 120 125

gcc ctg cag ccc acc cag ggt gcc atg ccg gcc ttc gcc tct gct ttc 432
 Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
 130 135 140

cag cgc cgg gca gga ggg gtc ctg gtt gct agc cat ctg cag agc ttc 480
 Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
 145 150 155 160

ctg gag gtg tcg tac cgc gtt cta cgc cac ctt gcg cag ccc 522
 Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
 165 170

<210> 2

<211> 174

<212> PRT

<213> Homo sapiens

<400> 2

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val

35 40 45

Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys

50 55 60

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser

65 70 75 80

Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser

85 90 95

Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp

100 105 110

Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro

115 120 125

Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe

130 135 140

Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe

145 150 155 160

Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170

<210> 3
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer for the N-terminal of hG-CSF

<400> 3
cgccgccata tgacaccct gggccctgcc ag 32

<210> 4
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer for the C-terminal of hG-CSF

<400> 4
accgaattcg gacctcagg gctgcgcaag gtggcg 36

<210> 5
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide for preparing E. coli enterotoxin II signal peptide

<400> 5

tcatgaaaaa gaatatcgca tttcttcttg catctatgtt cgttttttct attgctacaa 60

atgcctacgc gt

72

<210> 6

<211> 72

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing E. coli enterotoxin II signal peptide

<400> 6

acgcgtaggc attttagca atagaaaaaa cgaacataga tgcaagaaga aatgcgatat 60

tcittttcat ga

72

<210> 7

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for the N-terminal of [Ser1]hG-CSF

<400> 7

acaaatgcct acgcgtctcc cctgggccct gccagctcc

39

<210> 8

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for the C-terminal of [Ser1]hG-CSF

<400> 8

accgaattcg gatcctcagg gctgcgcaag gtggcgtaga ac 42

<210> 9

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for E.coli enterotoxin II Shine-Dalgarno sequence

<400> 9

cggtttccct ctgaggttg aggtgtttta tgaaaaagaa tatcgcatTT cttcttgcAT 60

ctatg 65

<210> 10

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide containing BamHI restriction site

<400> 10

accgaattcg gatcctcagg gctgcgcaag gtggcgtaga acgcg 45

<210> 11

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Last five amino acids of E. coli enterotoxin II signal peptide plus the 1st to the 5th amino acids of hG-CSF

<400> 11

Thr Asn Ala Tyr Ala Thr Pro Leu Gly Pro

1 5 10

<210> 12

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Thr1]hG-CSF

<400> 12

acaaatgcct acgcgacacc cctgggccct

30

<210> 13

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 12

<400> 13

agggcccagg ggtgtcgcgt aggcatttgt

30

<210> 14

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> N-terminal sequence of E. coli enterotoxin II signal peptide having threonine as the 4th amino acid

<400> 14

Met Lys Lys Thr Ile Ala Phe Leu

1

5

<210> 15

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for substituting the 4th amino acid of E. coli enterotoxin II signal peptide with threonine

<400> 15

ggtgttttat gaaaaagaca atcgatttc ttc

33

<210> 16

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID No: 15

<400> 16

gaagaaatgc gattgtcttt ttcataaaac acc

33

<210> 17

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> C-terminal sequence of E. coli enterotoxin II signal peptide having glutamine as the 22nd amino acid

<400> 17

Asn Ala Gln Ala Thr Pro Leu Gly

1

5

<210> 18

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for substituting the 22nd amino acid of E. coli enterotoxin II signal peptide with glutamine

<400> 18

caaatgccca agcgacaccc ctgggc

26

<210> 19

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 18

<400> 19

gcccaggggt gtcgcttggg catttg

26

<210> 20

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for modifying E. coli enterotoxin II Shine-Dalgarno sequence

<400> 20

tctagaggtt gagtgtttt atga

24

<210> 21

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 20

<400> 21

tcataaaaca cctcaacctc taga

24

<210> 22

<211> 66

<212> DNA

<213> Artificial Sequence

<220>

<223> S1 oligomer having E. coli-preferred nucleotide sequence coding for the 6th to 26th amino acids of [Ser17]hG-CSF

<400> 22

cagcctcttc tcttcacaa tctttccttc ttaagtctct tgaacaagtt agaaagatcc 60

aaggcg

66

<210> 23

<211> 66

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 22 (AS1 oligomer)

<400> 23

ccgggtcgga gaagagaagg tgtagaaag gaagaattca gagaacttgt tcaatctttc 60

taggtt

66

<210> 24

<211> 21

<212> PRT

<213> Escherichia coli

<220>

<221> SIGNAL

<222> (1).. (21)

<223> E. coli OmpA signal peptide

<400> 24

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala

1 5 10 15

Thr Val Ala Gln Ala
20

<210> 25

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide containing Hind III recognition site

<400> 25

gttgcgcaag cttctcga

18

<210> 26

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 25

<400> 26

tcgagaagct tgcgcaac

18

<210> 27

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for the N-terminal of [Ser1] hG-CSF

<400> 27

gttgcgcaag cttctcccct gggccctgcc agctccctg 39

<210> 28

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide containing EcoRI restriction site

<400> 28

accgaattct cagggctgcg caaggtggcg tagaacgcg 39

<210> 29

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> E. coli OmpA signal peptide plus the 1st to the 5th amino acids of [Ser1]hG-CSF

<400> 29

Gly Phe Ala Thr Val Ala Gln Ala Ser Pro Leu Gly Pro

1 5 10

<210> 30

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Thr1]hG-CSF

<400> 30

accgttgcg aagctacacc cctgggccct

30

<210> 31

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 30

<400> 31

agggccccagg ggtgtagctt gcgcaacggt

30

<210> 32

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Ser17]hG-CSF

<400> 32

agcttcctgc tcaagtcctt agagcaagtg agg

33

<210> 33

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 32

<400> 33

cctcacttgc tctaaagact tgagcaggaa gct 33

<210> 34

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Thr17]hG-CSF

<400> 34

agcttcctgc tcaagacctt agagcaagtg agg 33

<210> 35

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 34

<400> 35

cctcacttgc tctaaggtct tgagcaggaa gct 33

<210> 36

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Ala17]hG-CSF

<400> 36

agcttcctgc tcaaggcctt agagcaagtg agg

33

<210> 37

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 36

<400> 37

cctcacttgc tctaaggcct tgagcaggaa gct

33

<210> 38

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Gly17]hG-CSF

<400> 38

agcttcctgc tcaaggcctt agagcaagtg agg

33

<210> 39

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 38

<400> 39

cctcacttgc tctaagccct tgagcaggaa gct

33

<210> 40

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Asp17]hG-CSF

<400> 40

agcttcctgc tcaaggactt agagcaagtg agg

33

<210> 41

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 40

<400> 41

cctcacttgc tctaagtcct tgagcaggaa gct

33

<210> 42

<211> 18

<212> PRT

<213> Escherichia coli

<220>

<221> SIGNAL

<222> (1)..(18)

<223> E. coli Gene III signal peptide

<400> 42

Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser

1

5

10

15

His Ser

<210> 43

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide containing Nco I restriction site

<400> 43

tatagccata gcacatgga g

21

<210> 44

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 43

<400> 44

ctccatgggtg ctatggctat a

21

<210> 45

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> The 2nd to the 10th amino acids of hG-CSF

<400> 45

Pro Leu Gly Pro Ala Ser Ser Leu

1

5

<210> 46

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for the 2nd to the 10th amino acids of hG-CSF plus an additional cytosine at its 5'-end

<400> 46

ccccctgggc cctgccagct ccctg

25

<210> 47

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 46

<400> 47

cagggagctg gcagggccca ggggg

25

<210> 48
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> E. coli Gene III signal peptide plus the 1st to the 5th amino acids of hG-CSF

<400> 48

Phe Tyr Ser His Ser Thr Pro Leu Gly Pro

1 5 10

<210> 49

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> The 1st to the 9th amino acids of [Met2,Val3]hG-CSF

<400> 49

Thr Met Val Gly Pro Ala Ser Ser Leu

1 5

<210> 50

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Met2,Val3]hG-CSF

<400> 50

tacgcgtcca tggtagggccc tgccagctcc ctg

33

<210> 51

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 50

<400> 51

cagggagctg gcagggccca ccatggacgc gta

33

<210> 52

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> E. coli Gene III signal peptide plus the 1st to the 5th amino acids of
[Met2,Val3]hG-CSF

<400> 52

Phe Tyr Ser His Ser Thr Met Val Gly Pro

1

5

10

<210> 53

<211> 23

<212> PRT

<213> Escherichia coli

<220>

<221> SIGNAL
<222> (1)..(23)
<223> Thermoresistant E. coli enterotoxin II signal peptide

<400> 53
Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser
1 5 10 15

Ile Ala Thr Asn Ala Tyr Ala
20

<210> 54
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified thermoresistant E. coli enterotoxin II signal peptide

<400> 54
Met Lys Lys Thr Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser
1 5 10 15

Ile Ala Thr Asn Ala Gln Ala
20

<210> 55
<211> 96
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence coding for the 1st to 32nd amino acids of [Ser1, Ser17]hG-CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 55

tct ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48

Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

tct tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96

Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 56

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> the 1st to 32nd amino acids of [Ser1, Ser17]hG-CSF

<400> 56

Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 57

<211> 96

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Ser1]hG-CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 57

tct ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48

Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

tgc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 58

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> the 1st to the 32nd amino acids of [Ser1]hG-CSF

<400> 58

Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 59

<211> 96

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Ser17]hG-CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 59

aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

tct tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96

Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 60

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> the 1st to the 32nd amino acids of [Ser17]hG-CSF

<400> 60

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 61

<211> 96

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Thr17]hG--CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 61

aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

acc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96

Thr Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 62

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> the 1st to the 32nd amino acids of [Thr17]hG--CSF

<400> 62

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

Thr Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 63
<211> 96
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Ala17]hG-CSF

<220>
<221> CDS
<222> (1)..(96)

<400> 63
aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

gcc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
Ala Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

<210> 64
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> the 1st to the 32nd amino acids of [Ala17]hG-CSF

<400> 64
Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Ala Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 65
<211> 96
<212> DNA
<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for the 1st to the 32th amino acids of [Gly17]hG-CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 65

aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

ggc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96

Gly Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 66

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> the 1st to the 32th amino acids of [Gly17]hG-CSF

<400> 66

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

Gly Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 67

<211> 96

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Met2, Val3]hG-CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 67

aca atg gtc ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
 Thr Met Val Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

tgc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 68

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> the 1st to the 32nd amino acids of [Met2, Val3]hG-CSF

<400> 68

Thr Met Val Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

<210> 69
<211> 96
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Met2, Val3, Ser17]hG-CSF

<220>
<221> CDS
<222> (1)..(96)

<400> 69
aca atg gtc ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
Thr Met Val Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

tct tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

<210> 70
<211> 32
<212> PRT
<213> Artificial Sequence

<220>

<223> the 1st to the 32nd amino acids of [Met2, Val3, Ser17]hG-CSF

<400> 70

Thr Met Val Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

1 5 10 15

Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 30

<210> 71

<211> 10

<212> DNA

<213> Artificial Sequence

<220>

<223> Modified Shine-Dalgarno sequence

<400> 71

gaggtgtttt

10

<210> 72

<211> 96

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 57

<400> 72

ctggagcgct ggcctatcgc cctggatctt cctcacttgc tctaagcact tgagcaggaa 60

gctctggggc agggagctgg cagggcccag gggaga

96

<210> 73

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> E. coli OmpA signal peptide plus the 1st and the 2nd amino acids
of [Ser1]hG-CSF

<400> 73

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala

1

5

10

15

Thr Val Ala Gln Ala Ser Arg

20

<210> 74

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for the N-terminal of [Ser1] hG-CSF

<400> 74

gttgcgcaag cttctcccct gggccct

27

<210> 75

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 75

<400> 75

agggcccagg ggagaagctt gcgcaca

27

<210> 76

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for amino acids of SEQ ID NO: 48

<400> 76

ttctatagcc atagcacccc cctgggccct

30

<210> 77

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 76

<400> 77

agggcccagg ggggtgctat ggctatagaa

30

<210> 78

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for amino acids of SEQ ID NO: 52

<400> 78

ttctatagcc atagcaccat ggtgggcct 30

<210> 79

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 78

<400> 79

agggcccacc atggtgctat ggctatagaa 30